Indian Journal of Animal Sciences **91** (12): 1050–1053, December 2021/Short communication
https://doi.org/10.56093/ijans.v91i12.119821

Evaluation of lateral flow assay as a field test for sero-diagnosis of bovine brucellosis

JAYSUKH B KATHIRIYA[1]✉, NALINKANT M SHAH[2], BHAVESH J TRANGADIA[1], KIRIT R BHEDI[1], SAMIULLAKHAN H SINDHI[1] and SERENA MONTAGNARO[3]

Kamdhenu University, Junagadh, Gujarat 362 001 India

Received: 16 April 2021; Accepted: 29 September 2021

Keywords: Brucellosis, Bovine, Indirect ELISA, Lateral flow assay, RBPT

Brucellosis is an infectious disease caused by Gram negative facultative intracellular bacterial organisms of the genus *Brucella* which are pathogenic to a wide variety of animals and human beings. It is an emerging disease since the discovery of *Brucella melitensis* as the cause of Malta fever. The disease has a considerable impact on human and animal health and socio-economy as rural income relies largely on livestock breeding and dairy products in our country. Ever since the discovery of the causative agent, brucellosis remains one of the most important and widespread zoonosis world over causing significant morbidity and enormous economic losses due to infertility, delayed oestrus, interrupted lactation and loss of off-springs, wool, meat and milk production. Microbiological isolation and identification of the organisms is the gold standard test. But it is expensive, cumbersome and has a limited sensitivity (Ray 1979). Further, laboratory workers are at a great risk of catching the infection (Lopez-Merino 1991). Many serological tests and their modifications have been developed by various workers from time to time to detect antibodies against *Brucella* organism, viz. Rose Bengal plate test (RBPT), complement fixation test, milk ring test and enzyme-linked immunosorbent assay (ELISA). RBPT is routinely used sero diagnostic test for the brucellosis in our country and it is a quick, cheap, effective and OIE recognized test for the diagnosis of brucellosis. However, it has disadvantages of reporting false negative results due to prozone phenomenon.

Lateral flow assay (LFA) test was introduced for the first time in the Brucellosis Research laboratory of Bacterial Research Division, National Veterinary Research Institute, Vom, Plateau State, Nigeria in July 2009 (Bertu *et al.* 2010). LFA is simple, reliable, field based pen side diagnostic tool and does not require much of technical skill, refrigeration and specific equipment for the diagnosis of many infectious diseases including brucellosis (Smits *et al.* 1999, Shome *et al.* 2015, Kavya *et al.* 2017). It could be conveniently used in remotely located farms. Therefore, the present study was carried out to evaluate sensitivity and specificity of LFA and RBPT in comparison to indirect ELISA (iELISA) as gold standard test.

A total of 502 whole blood samples comprising 320 from cattle and 182 from buffaloes covering at least 10% of animals under flock were collected from farms/Gaushalas in districts of Southern Saurashtra region of Gujarat. Serum samples were separated and stored at –20°C until used. All these animals were above six months of age and none of these animals were vaccinated against brucellosis. The serum samples were subjected to RBPT, iELISA and LFA for the diagnosis of brucellosis.

Rose Bengal plate (RBPT) test antigen was procured from the Indian Veterinary Research Institute (IVRI), Izatnagar, Uttar Pradesh. The test was performed according to procedure described by the manufacturer. Briefly, 30 µl of serum was mixed with equal volume of *Brucella* antigen on white enamel plate circled approximately 2 cm in diameter with sterile glass or plastic rod. The result recorded after the mixture was rocked gently for 4 min at room temperature. Any sign of agglutination was considered as positive.

Indirect multi-species ELISA test kit (NovaTec VetLine Brucella, Germany) was used to screen these animals for detecting anti-brucella antibodies in serum. Before assaying, all samples were diluted 1:100 with sample diluent. 100 µl each of controls and diluted samples were dispensed into wells. The plate was incubated for 1 h at 37°C. After incubation the plate washed thrice with about 300 µl of washing buffer. Then 100 µl of VetLine Brucella Protein A/G conjugate was added to all micro wells except substrate blank well and incubated for 30 min at room temperature. After washing thrice, 100 µl of TMB substrate solution dispensed into all wells and incubated for 15 min at room temperature in dark. Finally, the reaction was stopped by adding 100 µl of stop solution in all the wells and plates were read on Thermo Scientific Multiskan GO Microplate Spectrophotometer at 450 nm filter to obtain optical density

Present address: [1]College of Veterinary Science and Animal Husbandry, Kamdhenu University, Junagadh, Gujarat. [2]College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, SK Nagar, Gujarat. [3]University of Naples Federico II, Via Delpino 1, Naples, Italy. ✉Corresponding author email: jbkathiriya@gmail.com

(OD) of the samples. The S/P% was calculated using the following formula:

$$\text{NovaTec units (NTU)} = \frac{\text{Sample or pool (mean) absorbance value}}{\text{Cut-off}} \times 10$$

The Cut-off is the mean absorbance value of the Cut-off control determinations. Samples with NTU<9 were classified as negative and NTU>11 were classified as positive, where as an NTU: 9–11 were classified as grey zone. The grey zone samples were subsequently retested by ELISA to classify either as negative or positive.

A commercial quick VET Bovine Brucella Ab lateral flow immunoassay kit (ubio Biotechnology Systems Pvt. Ltd., Cochin -Kerala) was used to screen animals for the presence of anti-brucella antibodies. Briefly, 10 µl of serum sample was added to sample well using a capillary tube and two drops of assay diluent were added over it. The test result was interpreted at 10 min. In negative sample, only control line (single line) appeared, while in positive sample two lines (control and test lines) were seen (Fig. 1).

The results of LFA and RBPT were compared with iELISA as gold standard because of its high specificity (Sp) and sensitivity (Se). Se and Sp of each test were calculated using MedCalc statistical software for Windows, version 19.3.1. Accuracies, Se and Sp of LFA and RBPT were statistically compared by McNemar's chi-square test using MedCalc software (MedCalc Software, Ostend, Belgium).

The diagnostic test should be simple, rapid and sensitive for regular screening of animals for brucellosis. RBPT is widely used test for the diagnosis of brucellosis. But, it often gives false positive results. ELISA has higher sensitivity and specificity but laboratory equipment and technical skills are required to perform the test. Hence, in the present study, LFA was compared with RBPT and iELISA as the gold standard test.

Fig. 1. Lateral flow assay. C, Control line; T, Test line.

Samples tested for RBPT, LFA and iELISA for cattle and buffaloes are shown in 2×2 table (Table 1). Out of 320 animals, 18.13%, 18.75%, 13.13% for cattle, while 182 animals 12.09%, 13.19%, 8.79% for buffaloes tested were positive by RBPT, iELISA and LFA, respectively. The sensitivity of RBPT vs iELISA was 81.67% and 87.50% and LFA vs iELISA was 68.33% and 66.67% in cattle and buffaloes, respectively. The specificity of RBPT and LFA was 96.54% and 99.62% in cattle, while 99.37% and 100.00% in buffaloes when compared with iELISA.

RBPT test showing higher sensitivity as compared to LFA, however specificity in both these test was comparable. The present findings supported the Se and Sp of RBPT and LFA to iELISA reported in cattle, sheep and goats by earlier workers (Khalek *et al.* 2012, El-Eragi *et al.* 2014, Elshemey and Abd-Elrahman 2014, Kotadiya *et al.* 2015, Kavya *et al.* 2016, Saadat *et al.* 2017). Ahmed *et al.* (2016) reported a lower Se and Sp for both these tests. However, Trangadia and Prasad (2017) recorded lower Se and Sp of RBPT vs iELISA, while comparable Se and Sp of LFA vs iELISA in goats as compared to present findings. Higher Se and Sp of RBPT and LFA were reported by Rahman *et al.* (2013) and Hota *et al.* (2016). Conversely, Guci *et al.* (2019) reported highest diagnostic Se of RBT as compared to LFA and iELISA in cattle.

The negative predictive value (NPV) for RBPT was 95.80% and 98.12% while 93.17% and 95.21% for LFA in cattle and buffaloes, respectively. However, the positive predictive values (PPV) for RBPT was 84.48% and 95.45% while 97.62% and 100.00% for LFA in cattle and buffaloes, respectively. McNemar chi-square test for independent data (with Yates' correction) revealed significant difference in the positive proportion between RBPT vs iELISA as 0.62% and 1.10%, while LFA vs iELISA as 5.62% and 4.40% in cattle and buffaloes, respectively. The concordance of iELISA with RBPT was (k=0.792 and k=0.900), while (k=0.811 and k=0.776) for LFA in cattle and buffaloes, respectively (Table 2).

Shome *et al.* (2015) observed lower PPV and NPV values as compared to the present study during their study at organized buffalo farm. However, Trangadia and Prasad (2017) and Hota *et al.* (2016) reported comparable values for PPV and NPV in goats and bovines, respectively. Kappa values recorded in present study supported the findings by earlier workers (El-Eragi *et al.* 2014, Elshemey and Abd-Elrahman 2014, Kushwaha *et al.* 2015, Kavya *et al.* 2016) but could not support kappa values reported by Ahmed *et al.* (2016) which was comparatively lower than ours. Higher kappa values were also reported by some of the workers in past (Hota *et al.* 2016, Kushwaha *et al.* 2016).

Table 1. Comparison of LFA and RBPT with iELISA for cattle and buffaloes (2 × 2 Table)

RBPT vs iELISA (Total 320 samples) for cattle					
Test (RBPT)	Positive by iELISA	n	Negative by iELISA	n	Total
Positive	True positive	a = 49	False positive	c = 09	a+c = 58
Negative	False negative	b = 11	True negative	d = 251	b+d = 262
Total		a+b = 60		c+d = 260	
LFA vs iELISA (Total 320 samples) for cattle					
Test (LFA)	Positive by iELISA	n	Negative by iELISA	n	Total
Positive	True positive	a = 41	False positive	c = 01	a+c = 42
Negative	False negative	b = 19	True negative	d = 259	b+d = 278
Total		a+b = 60		c+d = 260	
RBPT vs iELISA (Total 182 samples) for buffaloes					
Test (RBPT)	Positive by iELISA	n	Negative by iELISA	n	Total
Positive	True positive	a = 16	False positive	c = 00	a+c = 16
Negative	False negative	b = 06	True negative	d = 160	b+d = 166
Total		a+b = 22		c+d = 160	
LFA vs iELISA (Total 182 samples) for buffaloes					
Test (LFA)	Positive by iELISA	n	Negative by iELISA	n	Total
Positive	True positive	a = 16	False positive	c = 00	a+c = 16
Negative	False negative	b = 08	True negative	d = 159	b+d = 167
Total		a+b = 24		c+d = 159	

RBPT, Rose Bengal plate test; iELISA, indirect enzyme-linked immunosorbent assay; LFA, lateral flow assay; a, number of samples positive to both conventional and the standard tests; b, number of samples negative to conventional but positive to the standard test; c, number of samples positive to conventional but negative to the standard test; d, number of samples negative to both conventional and the standard tests; n, number of samples.

Table 2. Evaluation of RBPT and LFA in comparison with iELISA

Statistic	RBPT		LFA	
	Cattle	Buffaloes	Cattle	Buffaloes
Sensitivity (95% CI)	81.67% (69.56–90.48%)	87.50% (67.64–97.34%)	68.33% (55.04–79.74%)	66.67% (44.68–84.37%)
Specificity (95% CI)	96.54% (93.53–98.41%)	99.37% (96.52–99.98%)	99.62% (97.88–99.99%)	100.00% (97.71–100.00%)
Positive predictive value (95% CI)	84.48% (73.92–91.27%)	95.45% (74.74–99.33%)	97.62% (85.19–99.66%)	100.00%
Negative predictive value (95% CI)	95.80% (93.04–97.50%)	98.12% (94.78–99.34%)	93.17% (90.38–95.19%)	95.21% (91.86–97.22%)
Kappa statistics (95% CI)	0.792 (0.704–0.880)	0.900 (0.804–0.997)	0.811 (0.721–0.901)	0.776 (0.625–0.928)
Mc Nemar test Difference (95% CI)	0.62% (–2.31–3.36%)	1.10% (–1.30–2.02%)	5.62% (3.13–6.15%)	4.40% (1.14–4.40%)
Chi-square	0.0500	0.2500	14.4500	6.1250
Significance	P=0.8231	P=0.6171	P=0.0001	P=0.0133

RBPT, Rose Bengal Plate Test; iELISA, indirect enzyme-linked immunosorbent assay; LFA, lateral flow assay; CI, confidence interval.

SUMMARY

Indirect-ELISA offers a significant advantage over conventional serological methods in the diagnosis of brucellosis in endemic geographical region. Considering iELISA as a gold standard test, RBPT was more sensitive than LFA and the concordance of iELISA with LFA was comparable. The Lateral flow assay is a rapid point-of – care diagnostic test which makes it ideal for use in resource pour countries. It is an immuno-assay and is used also for diagnosis of bovine brucellosis. It is a highly sensitive and specific test which does not require expensive equipment,

electricity and or refrigeration or special training. The LFA has shown good PPV (positive predictive value) and NPV (negative predictive value) greater than RBPT in the current study suggest that the test is a simple, cost-effective and rapid that provides accurate detection of antibodies to *B. abortus* in bovine serum samples, thereby saving time and eliminating the need for special training. It could be used conveniently on the field even in farms located in remote areas. However, evaluation on large sample size would be required for future use.

Hence, looking to the results obtained in the present study and by other workers, it is recommended that this rapid test can therefore be practically implemented in serological screening for bovine brucellosis, although evaluation on a larger scale with various sera, and blood samples is still necessary.

ACKNOWLEDGEMENT

Authors are thankful to In-charge Head, Department of Veterinary Mcrobiology; Dean, College of Veterinary Science and Animal Husbandry, Junagadh Agricultural University, Junagadh, Gujarat (India) and Management of various farms and gaushalas for their help and cooperation in carrying out this study.

REFERENCES

Ahmed W A, Majeed S A, Abdul Ameer A H, Mahmmod N D, Saeed N I and Hanna L Y. 2016. Sensitivity and specificity of various serological tests for detection of *Brucella* spp. infection in male goats and sheep. *Advances in Microbiology* **6**: 98–103.

Bertu W J, Ocholi R A, Gusi A M, Mwankon E and Hassan M. 2010. Brucella lateral flow assay: A newly introduced test for rapid diagnosis of brucellosis. *Vom Journal of Veterinary Science* **7:** 42–46.

El-EragiA M, Salih M H, Alawad M F E M and Mohammed K B. 2014. Evaluation of lateral flow assay for serodiagnosis of bovine brucellosis in Gezira State, Sudan. *Veterinary World* **7**: 395–97.

Elshemey T M and Abd-Elrahman A H. 2014. Evaluation of a rapid immuno-chromatographic test for detection of *Brucellaabortus*antibodies in Egyptian cattle sera and milk. *Alexandria Journal of Veterinary Sciences* **40**: 24–28.

Gusi A M, Bertu W J, Jesús de Miguel M, Dieste-Pérez L, Smits H L, Ocholi R A *et al.* 2019. Comparative performance of lateral flow immunochromatography, iELISA and Rose Bengal tests for the diagnosis of cattle, sheep, goat and swine brucellosis. *PLOS Neglected Tropical Diseases* **13**(6): e0007509.

Hota S R, Sahoo N and Satpathy S. 2016. Comparison of RBPT, STAT and rapid lateral flow lateral flow assay against indirect ELISA for diagnosis of bovine brucellosis. Proceedings of International Brucellosis Research Conference, New Delhi, India. pp. 134

Kavya B A, Veeregowda B M, Isloor S, Gowda L, Kamran C, Gomes A R, Triveni K, Padmashree B S and Shome R. 2017. Comparative evaluation of blood based lateral flow assay for diagnosis of brucellosis in livestock species. *Indian Journal of Animal Sciences* **87**(9): 1068–70.

Kavya B A, Veeregowda B M, Shome R, Gowda L, Suresh K P and Gajendragad M R. 2016. A pilot study evaluation of lateral flow assay – a point of care diagnostic for brucellosis. Proceedings of International Brucellosis Research Conference, New Delhi, India. pp.135

Khalek M M A, Ramadan K M, Hazem S S and Khairy E A. 2012. Evaluation of lateral flow assay for serodiagnosis of *Brucella* among cattle, sheep and goats in Egypt. *Global Veterinaria* **8**: 511–18.

Kotadiya A J, Kumar P, Patel K M, Patel M V, Shroff S I, Dadawala A I, Srimali M D, Bhagat A, Chandel B S and Shah N M. 2015. Seroprevalence of ovine brucellosis in North Gujarat and Kutchh regions of Gujarat by i-ELISA and its comparison with RBPT and STAT. *Indian Veterinary Journal* **92**: 42–46.

Kushwaha N, Rajora V S, Mohan A and Gupta T K. 2015. Comparative efficacy of diagnostics used for detection of *Brucella* antibodies in cattle. *Indian Veterinary Journal* **92**: 18–20.

Lopez-Merino A. 1991. Brucelosis. Progress and Perspectives. Technical Publication of INDRE-SSA No. 6. Secretariat of Health, Mexico City. pp. 54.

Rahman A K, Saegerman C, Berkvens D, Fretin D, Gani M O, Ershaduzzaman M, Ahmed M U and Emmanuel A. 2013. Bayesian estimation of true prevalence, sensitivity and specificity of indirect ELISA, Rose Bengal test and slow agglutination test for the diagnosis of brucellosis in sheep and goats in Bangladesh. *Preventive Veterinary Medicine* **110**: 242–52.

Ray W C. 1979. Brucellosis (due to *Brucella abortus* and *B. suis*). Hand Book Series in Zoonosis. (Ed) Stell J H. Vol. 1, CRC Press Inc., Florida. pp. 99–185.

Saadat S, Mardaneh J, Ahouran M, Mohammadzadeh A, Ardebili A, Yousefi M and Mansouri M. 2017. Diagnosis of cattle brucellosis by PCR and serological methods: Comparison of diagnostic tests. *Biomedical and Pharmacology Journal* **10**: 881–88.

Shome R, Filia G, Padmashree B S, Krithiga N, Sahay S, Triveni K, Shome B R, Mahajan V, Singh A and Rahman H. 2015. Evaluation of lateral flow assay as a field test for investigation of brucellosis outbreak in an organized buffalo farm: A pilot study. *Veterinary World* **8**: 492–96.

Smits H L, Basahi M A, Diaz R, Marrodan T, Douglas J T, Rocha A, Veerman J, Zheludkov M M, Witte O W M, De Jong J, Gussenhoven G C, Goris M G A and Vanderhoorn M A W G. 1999. Development and evaluation of rapid dipstick assay for the serodiagnosis of the early phase of brucellosis in humans. *Journal of Clinical Microbiology* **37**: 4179–82.

Trangadia B J and Prasad M C. 2017. Evaluation of immunochromatographic assay as a field test for the diagnosis of caprine brucellosis. *Indian Journal of Animal Sciences* **88**(8): 877–80.